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Prenatal and pubertal testosterone affect brain lateralization

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ABSTRACT

After decades of research, the influence of prenatal testosterone on brain lateralization is still elusive, whereas the influence of pubertal testosterone on functional brain lateralization has not been investigated, although there is increasing evidence that testosterone affects the brain in puberty. We performed a longitudinal study, investigating the relationship between prenatal testosterone concentrations in amniotic fluid, pubertal testosterone concentrations in saliva, and brain lateralization (measured with functional Transcranial Doppler ultrasonography (fTCD)) of the Mental Rotation, Chimeric Faces and Word Generation tasks. Thirty boys and 30 girls participated in this study at the age of 15 years. For boys, we found a significant interaction effect between prenatal and pubertal testosterone on lateralization of Mental Rotation and Chimeric Faces. In the boys with low prenatal testosterone levels, pubertal testosterone was positively related to the strength of lateralization in the right hemisphere, while in the boys with high prenatal testosterone levels, pubertal testosterone was negatively related to the strength of lateralization. For Word Generation, pubertal testosterone was negatively related to the strength of lateralization in the left hemisphere in boys. For girls, we did not find any significant effects, possibly because their pubertal testosterone levels were in many cases below quantification limit. To conclude, prenatal and pubertal testosterone affect lateralization in a task-specific way. Our findings cannot be explained by simple models of prenatal testosterone affecting brain lateralization in a similar way for all tasks. We discuss alternative models involving age dependent effects of testosterone, with a role for androgen receptor distribution and efficiency.

1. Introduction

Brain lateralization is the functional specialization of the brain, with some functions performed primarily by the left hemisphere, and other functions by the right hemisphere. This is a basic organizational principle of the brain throughout the animal kingdom, with important consequences for behavior, perception and cognitive processes. In humans, there is considerable individual variation in how functions are divided over the two hemispheres (Pujol et al., 1999; Lust et al., 2011a), but the developmental trajectory of these differences in lateralization remains elusive. Prenatal testosterone has been put forward as a major causal factor in the development of brain lateralization, inspired by sex differences in lateralization of brain and behavior (Springer and Deutsch, 1997, but see Pfannkuche et al., 2009), in combination with the fact that prenatal exposure to testosterone plays a major role in sexual differentiation of the brain (Arnold and Breedlove, 1985; Cooke et al., 1998). However, more recently it has become evident that puberty is also an important developmental phase in which testosterone can have organizational effects on brain and behavior (Blakemore et al., 2010; Peper and Dahl, 2013; Romeo, 2003; Sisk and Zehr, 2005). This aspect has been neglected in studies on brain lateralization.

There are three theories on the effect of prenatal testosterone on brain lateralization: the Sexual Differentiation theory is based on observed differences in lateralization between males and females, but makes no assumptions on the underlying mechanisms (Hines and Shipley, 1984). Males are more often left-handed (Annett, 1985), which led to the hypothesis that males are more asymmetrically lateralized, although the effect size is small. The Geschwind and Galaburda theory proposes that prenatal testosterone delays growth of brain areas in the left hemisphere, resulting in compensatory growth of the homologue

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regions in the right hemisphere (Geschwind and Galaburda, 1985). This would result in a weaker lateralization of functions lateralized in the left hemisphere, and a stronger lateralization of functions lateralized in the right hemisphere. The Corpus Callosum theory is based on correlational evidence in males, suggesting that prenatal testosterone induces pruning of the corpus callosum (Witelson and Nowakowski, 1991), which is the main connection between the two hemispheres. Prenatal testosterone would reduce crosstalk between the hemispheres, and would thereby promote execution of a function within one hemisphere. Chura et al. (2010) measured prenatal testosterone levels in amniotic fluid in humans, and found that prenatal testosterone is positively related to rightward asymmetry of the isthmus of the corpus callosum at age 8–11 years. Interestingly, the isthmus projects to brain regions involved in lateralized functions such as language, emotion recognition and visuospatial cognition (Chura et al., 2010).

After decades of research, the role of prenatal testosterone in the development of brain lateralization is still unclear (see review (Pfannkuche et al., 2009)). This may partly be caused by the fact that most studies estimate individual prenatal testosterone levels indirectly, for example by the 2D:4D finger length ratio (Cohen-Bendahan et al., 2005; for a critique see Beking et al., 2017). In the present study, prenatal testosterone is measured in amniotic fluid, via amniocentesis. Amniocentesis is the most direct way to measure prenatal testosterone exposure in humans. It is performed between week 15–18 of gestation, this is around the time that the maximum sex difference in testosterone levels occurs (Abramovich, 1974), and during the sensitive period (week 8–24 of gestation) in which prenatal hormones influence sexual differentiation of the brain (Knickmeyer and Baron-Cohen, 2006). There are only three studies that have measured amniotic testosterone levels to investigate its relation with functional lateralization later in life. Grimshaw et al. (1995) found that prenatal testosterone is positively correlated with lateralization of language (dichotic listening) and handedness in the left hemisphere in girls, and with lateralization of recognizing emotions (dichotic listening of emotional affect) in the right hemisphere in boys at age 10. Lust et al. (2010) found that prenatal testosterone is positively correlated with the strength of lateralization of language (dichotic listening) in children aged 6 years. The only study that measured lateralization at the brain level (with EEG), instead of at the performance level, found no relation between prenatal testosterone and lateralization of language and face processing in boys of 7–10 years old (Mercure et al., 2009). Thus, the results are mixed.

The effects of hormones are classified into “organizing effects” and “activating effects”, although the distinction between the effects is not absolute (Arnold and Breedlove, 1985). Historically, organizing effects were only considered to happen during the prenatal period, but there is increasing evidence that hormones later in life could have organizing effects on the brain as well, particularly during puberty (Blakemore et al., 2010; Peper and Dahl, 2013; Romeo, 2003; Sisk and Zehr, 2005). That is, just like prenatal testosterone, pubertal testosterone can have structural and permanent effects on the brain, which can result in sex differences (Giedd et al., 2012; Peper et al., 2011; Peper et al., 2009; Perrin et al., 2008; Raznahan et al., 2010). Thus, if we extend the sexual differentiation theory of Hines and Shipley (1984) to pubertal testosterone, we hypothesize that pubertal testosterone could also affect brain lateralization.

Literature investigating the influence of pubertal hormones on lateralization is limited to menstrual cycle effects in girls, and finds that estradiol and progesterone temporarily seem to reduce cerebral asymmetries (e.g. Hausmann and Güntürkün 2000; Hjelmervik et al., 2012; Hodgetts et al., 2015). These studies investigate the activating effects of fluctuating hormone levels. Remarkably, there are no studies on the relation between testosterone and lateralization in puberty, but studies on testosterone exposure later in life are limited to adulthood. A recent study found that higher testosterone levels in adulthood were accompanied by stronger lateralization of Word Generation measured with functional Transcranial Doppler (the same technique that we used) (Papadatou-Pastou and Martin, 2017), but a review of behavioural studies on language lateralization did not find consistent relations with adult testosterone levels (Papadatou-Pastou et al., 2016). There is clearly a need for studies which (1) investigate the effects of both prenatal and pubertal testosterone longitudinally, including the potential interaction effect, (2) are based on direct measurements of the hormone concentrations, (3) analyse lateralization directly at the brain level.

In the current paper we present the results of a longitudinal study analysing the relationships between prenatal and puberal testosterone levels and brain lateralization of cognitive functions known to be lateralized. This study is a follow-up of the studies by Lust et al. (2010, 2011c), who found a positive correlation between prenatal testosterone and language lateralization in children at 6 years of age. These children have now reached the age of puberty, which allows us to study the effects of pubertal testosterone as well. The other main difference with the previous study is that we used a more direct measurement of brain lateralization by means of functional Transcranial Doppler (fTCD) ultrasonography. FTCD measures the change blood flow velocity in the left and the right hemisphere (in the middle cerebral arteries) during a cognitive task. The fTCD technique is based on the assumption that blood flow velocity increases after a hemisphere becomes more active. With fTCD we assessed lateralization of three cognitive tasks, enabling comparison of outcomes between different functions. Word Generation and Mental Rotation are strongly lateralized tasks (respectively to the left and right hemisphere), and validated for fTCD (Stroobant and Vingerhoets, 2000). The Chimeric Faces task is often used as indirect measure of lateralization of emotional face processing, in which sex differences in lateralization have been found (e.g. Bourne, 2005). We used fTCD with this task for the first time.

The three theories on the influence of prenatal testosterone on brain lateralization differ in their predictions with regard to the effect on brain lateralization, and support from the literature for these is inconsistent. Therefore, we refrain from clear hypotheses. We do expect that pubertal testosterone has a similar effect to that of prenatal testosterone, as both affect sexual differentiation in brain and behavior, the inspiration for assuming an effect of androgens on brain lateralization. Moreover, based on the classic idea that prenatal testosterone sensitizes the brain via increased receptor densities for testosterone later in life (Nelson, 2005), we expect an interaction effect between prenatal and pubertal testosterone on brain lateralization such that high prenatal testosterone levels strengthen the effects of testosterone during puberty.

2. Method

2.1. Participants

Thirty boys (mean age $M = 15.0$, $SD = 0.60$, range [14.0–16.1]) and 30 girls (mean age $M = 15.06$, $SD = 0.58$, range [14.0–16.1]) were invited from an initial sample of 196 children born in 2000, whose mothers underwent amniocentesis. Genotyping of the amniotic fluid samples was performed, and all boys were XY and all girls XX. No differences were observed in handedness between boys and girls. Twenty-four boys and 22 girls were right-handed, 3 boys and 3 girls were left-handed, and 3 boys and 5 girls were ambidexter (see paragraph 2.4). Due to technical problems with the fTCD measurement two

right-handed girls were excluded from the analysis. Ethical clearance and consent was given.

2.2. Procedure

In the first part of the study, participants completed an online anonymous questionnaire including questions to determine stage of puberty and handedness. In the second part of the study, author TB visited the home of the participant for the fTCD-measurement of brain lateralization (duration 50 min), and the collection of saliva samples for hormone analyses.

2.3. Puberty assessment

Boys indicated their stage of puberty by reporting one or more of the following characteristics: presence of pubic hair, growth spurt, voice cracking, deepening of voice, if they shaved their face, if they have had a first ejaculation, or none of the above. These characteristics were selected after consultation with a medical specialist in child and adolescent endocrinology with experience in assessing pubertal stage (for literature on pubertal stages in boys see Tanner, 1962). Pubertal stage is calculated as the sum of these characteristics (1 point per characteristic; M = 4.1, SD = 1.6). If the boys indicated that they had a first ejaculation, they were asked to specify the date of their first one (days since first ejaculation M = 503, SD = 489, range [0-1755]). Minimal variation in stage of puberty was expected in girls, with almost all girls having reached the last stage at 15 years of age (Marshall and Tanner, 1969; and based on the advice of the medical specialist). Menarche occurs at the end of puberty, and girls were asked to specify the date of their first menstruation (days since first menstruation M = 652, SD = 403, range [0–1959]).

2.4. Handedness

Handedness was determined with the Dutch translation of the Edinburgh Handedness Inventory (Van Strien, 2002). The online questionnaire consisted of 11 questions and scores ranged from −100 ("always with left hand") to 100 ("always with right hand") per question. Participants with a total score of > 800 were classified as right-handed, < −800 as left-handed, and the rest as ambidexter (Van Strien, 2002).

2.5. Prenatal testosterone (amniotic fluid)

Amniocentesis was performed in the 15–18th week of pregnancy at the University Medical Center of Utrecht, the Netherlands. The reason for amniocentesis was age of the mother (36–42 years). Testosterone levels were measured in the amniotic fluid by radioimmunoassay. For more information, including exclusion criteria for further analyses, please see (Van de Beek et al., 2004).

2.6. Pubertal testosterone (saliva)

Testosterone levels were assessed in saliva. The participants were asked to produce 4 ml of saliva via passive drooling through a polypropylene straw (Durdiaková et al., 2013). On the test day two samples were taken (sample 1 M_{time} = 14:00, sample 2 M_{time} = 14:30, range [12:15–17:20]). Two weeks after the visit two more samples were taken. However, we only used sample 1 for our analysis, as the correlation between the four samples was high (between sample 1 and 2: $r = 0.98$; between sample 1 and 3: $r = 0.87$; and between sample 1 and 4: $r = 0.84$; all $p < 0.001$), and because we were primarily interested in the pubertal testosterone levels close to the moment the lateralization index was determined.

Testosterone in saliva was analyzed by isotope dilution liquid chromatography tandem mass spectrometry (LC–MS/MS). Imprecision at 0.13 nmol/L was 3.1% (repeatedly measured at 14 days). Lower limit of quantification for testosterone was 0.01 nmol/L. Twenty out of 30 girls had pubertal testosterone levels below the lower limit of quantification. Testosterone levels were within the range of the reference values measured by Bui et al. (2013). Prenatal and pubertal testosterone levels differed in magnitude because they were assessed in different types of samples (amniotic fluid or saliva) and with a different technique (radioimmunoassay or LC–MS/MS).

2.7. Functional transcranial doppler (fTCD)

Brain lateralization was measured with functional Transcranial Doppler ultrasonography (fTCD). Using a DWL Doppler Box (Compumedics Germany GmbH) and QL 3.0 software, we measured the blood flow velocity in the left and in the right middle cerebral arteries simultaneously during a cognitive task and during baseline. The middle cerebral arteries supply most of the cortex of blood. FTCD is based on the assumption that greater activity in a hemisphere during a task requests larger blood flow in the corresponding middle cerebral artery. For example, if the right hemisphere is dominant for Mental Rotation, the blood flow velocity will increase more in the right than in the left hemisphere compared to the baseline. Results of this technique correlate very well with fMRI results (Deppe et al., 2000; Jansen et al., 2004). A cap with 2 probes (2-Mhz transducers) is placed on the head of a participant, on the left and the right temporal bone window. Both probes emit a high-pitched sound signal that reflects off the blood cells in the left and right middle cerebral artery. The faster the blood flows, the bigger the Doppler shift of the reflected signal. For more information on the fTCD procedure see (Bishop et al., 2010; Deppe et al., 2004).

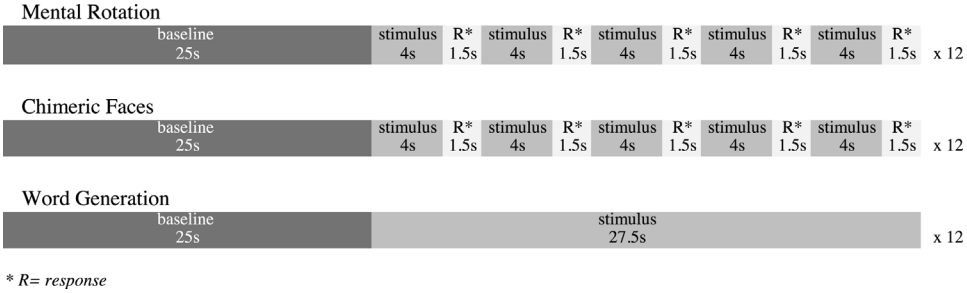

Fig. 1. Task design.

2.8. Tasks

Brain lateralization was measured during the performance of Mental Rotation, Chimeric Faces, and Word Generation tasks. The tasks were performed on a laptop (HP Pro Book), and experimental paradigms controlled by E-prime 22. The task order was randomly assigned. Every task consisted of 12 trials, and started with one (Chimeric Faces and Word Generation) or two (Mental Rotation) practice trials. Every trial lasted 52.5s, consisting of a baseline (25s) and an active task (27.5s), see Fig. 1. The baseline was always a white screen. During Mental Rotation and Chimeric Faces, the participants were instructed to respond with a press on a button box with 2 buttons with both index fingers simultaneously, to minimize lateralized activation in the motor cortex.

2.8.1. Mental rotation

During the Mental Rotation task, a pair of 3D-figures was shown to the participant for 4 s (see Fig. A.1 in Appendix B for an example). The participant was instructed to decide whether the figures are identical or mirrored (50% chance). Next, a white screen was shown for 1.5s, during which the participant had to respond by pressing the upper button in case both figures were identical, and the lower button in case the figures were mirrored. Every trial consisted of 5 stimulus pair-response sequels. The stimuli used were similar to those used in (Vandenberg and Kuse, 1978).

2.8.2. Word generation

The participant was instructed to whisper as many words as possible that begin with a given letter. The participant was instructed not to use numbers, non-existing words, names and “compound words” starting with a previous word. The task started with one practice trial (letter: S). After the baseline period, a single letter was shown for 27.5s, after which 12 trials followed (letters: J, T, N, B, E, O, W, R, M, P, F, K).

2.8.3. Chimeric faces

During the Chimeric Faces tasks a pair of two identical but mirrored faces was shown to the participant in vertical orientation, with an emotion (happy or angry) expressed on the left or right side of the face, and the other half showing a neutral expression (see Fig. A.2 in Appendix B). The participant was instructed to indicate which face looked more emotional. First, the stimulus pair was shown for 4s. Next, a white screen was shown for 1.5 s during which the participant had to respond by pressing the upper button if he/she thought the upper face looked more emotional, and the lower button for the lower face. Every trial consisted of 5 different stimulus pairs with the same emotion. The trials were randomized for happy and angry emotion (each 50%), and for position (upper/lower, each 50%). For more information on the Chimeric Faces stimuli, and a link to the stimulus set we created, see Text A.1 in Appendix A.

2.9. Measurement of laterality

The Doppler signal (measured with fTCD) was processed with the dopOSCCI 2 software package (Badcock et al., 2012) for Matlab (R2011b). In dopOSCCI we chose the following settings: (1) epochs with 30% lower or higher blood flow velocity than average were excluded, (2) epochs with a left-right blood flow velocity difference of more than 20% relative to the normalized signal were excluded, (3) data was normalized using an epoch to epoch method. The baseline period for the analysis was fixed at minus 15 s to onset of the stimulus. The period of interest was set to 7–27.5 s after onset of the stimulus. Participants with less than 7 accepted epochs were excluded from the analysis: 1 girl was excluded for the Mental Rotation task (n = 57), 1 girl and 1 boy for the Chimeric Faces task (n = 56), and 1 boy for the Word Generation task (n = 57).

The lateralization index (LI) was calculated from the difference in blood flow between task and preceding baseline for the left (ΔL) and right hemisphere (ΔR) as follows: LI = (ΔL) − (ΔR). Thus, a positive lateralization index indicates lateralization to the left hemisphere, and a negative index lateralization to the right hemisphere. As the lateralization index derived from fTCD is a relative measure, we cannot say if a stronger positive LI means that the left hemisphere is more activated, or that the right hemisphere is less activated compared to baseline, or both, and vice versa.

In published fTCD studies, the LI is based on a period of only 2 s around the maximum difference between left and right activation. Using the AVERAGE software available at that time (Deppe et al., 1997), this was the only way to determine the LI. With the recent dopOSCCI software the LI can be calculated over the entire period of interest. We chose to do this, as we are interested in the LI during the entire task period. Moreover, the latency of the maximum difference varied greatly over the entire task period, and could even reverse in sign, making the LI based on only 2 s around the maximum a less reliable measure. The standard deviation of the LI is smaller when the entire LI is used, and fewer epochs were rejected. Therefore, the results based on the LI of the entire period of interest are presented in this article. To enable comparison with other fTCD studies, the outcomes based on the maximum difference are presented in Table A.1 in Appendix B.

2.10. Statistical analysis

SPSS 22 was used for the statistical analyses. An independent samples *t*-test was performed to test sex differences for all variables. Prenatal and pubertal testosterone were not normally distributed, therefore a Mann-Whitney U Test for these variables was applied. Normally, in fTCD studies, the average LI over all trials is used in the analysis. However, the dopOSCCI software provided us with the LI of every trial, and we used a linear mixed model to control for the variance between the trials of the same person (covariance type: Scaled Identity; Sum of Squares Type III; Estimations based on the Maximum Likelihood). First, an analysis with only prenatal testosterone and sex as fixed effects, and subject as random effect, was performed including all participants. Secondly, since the overlap in pubertal testosterone levels between boys and girls turned out to be minimal, we performed further analyses for each sex separately.

We calculated the correlation between duration of puberty (pubertal stage for boys, and days since first menstruation for girls) and pubertal testosterone. To control for the duration of exposure to elevated pubertal testosterone levels in boys, we added pubertal as a covariate to the model. Based on Akaike’s criterion (AIC) the model with pubertal stage was better than the model without it. However, to be able to also directly compare the outcomes between boys and girls, an additional analysis was performed for boys without pubertal stage as a covariate. Visual inspection revealed that the residuals of all models were normally distributed. In addition, to control for the effect of time of day of saliva collection on pubertal testosterone levels, all analyses were performed again with time of day added as a covariate. The results did not change qualitatively and based on the AIC the model was better without time of sampling as a covariate. Therefore, the results will be

Table 1
Mean (M) and standard deviation (SD) of all variables, for boys and girls.

	Boys		Girls		sex-effect	
	M	SD	M	SD	test value*	*p*
Age (years)	15.0	0.6	15.0	0.6	0.27	0.785
Prenatal testosterone (nmol/L)	1.50	0.55	0.71	0.39	6.34	< **0.001**
Pubertal testosterone (nmol/L)	0.12	0.07	0.015	0.011	7.32	< **0.001**
LI Mental Rotation	−1.97	1.74	−1.00	1.91	−2.01	**0.049**
LI Chimeric Faces	−2.14	1.85	−1.63	1.86	−1.03	0.308
LI Word Generation	1.32	1.65	1.08	2.04	0.50	0.622

*Significance (p) of the sex-effect was tested with a Mann-Whitney *U* test for prenatal and pubertal testosterone and a *t*-test (df = 56) for the other variables.

presented without time of saliva collection as a covariate. Also, all analyses were performed on the right-handed participants only (see Table A.2 in Appendix B), revealing qualitatively the same results.

To interpret the interaction effects of prenatal and pubertal testosterone on the Mental Rotation and Chimeric Faces tasks, the model was run again, but this time with the standardized z-scores of all independent variables. Because only the interaction effects remained significant, we focused on these. To visualize the interaction between prenatal and pubertal testosterone, the boys were divided in a "low prenatal testosterone group" and a "high prenatal testosterone group" based on the median split, and the relation between pubertal testosterone levels and brain lateralization was graphically depicted for these two groups. Additional analyses (linear mixed model) were performed to assess the effect of pubertal testosterone and pubertal stage on lateralization for both prenatal groups. The Beta-values of all effects were calculated as the B-values of the mixed model with the standardized z-scores of all independent variables.

3. Results

3.1. Prenatal and pubertal testosterone

Prenatal testosterone concentrations in amniotic fluid were significantly higher in boys than in girls (Table 1), with some overlap (see Fig. A.3 in Appendix B). Boys also had significantly higher concentrations of testosterone in saliva during puberty (Table 1), but here the overlap between the sexes was minimal with 20 out of 30 girls having pubertal testosterone levels under the detection threshold of 0.01 nmol/L (see Fig. A.3 in Appendix B). The correlation between prenatal and pubertal testosterone levels was not significant (boys: Spearman's $r = 0.15$; $p = 0.440$; girls: Spearman's $r = -0.14$; $p = 0.466$; see Fig. A.3 in Appendix B). For boys, the correlation between pubertal testosterone and pubertal stage was significant (Spearman's $r = 0.50$; $p = 0.005$; see Fig. A.4 in Appendix B). For girls, there was no correlation between days since first menstruation and pubertal testosterone level (Pearson's $r = -0.07$, $p = 0.710$).

3.2. Lateralization of the mental rotation, chimeric faces and word generation tasks

Both the Mental Rotation task and the Chimeric Faces task were significantly lateralized to the right hemisphere (Mental Rotation: $M = -1.51$, $SD = 1.87$; Chimeric Faces: $M = -1.90$; $SD = 1.85$), and the Word Generation task to the left hemisphere ($M = 1.20$; $SD = 1.84$). The average activation during the tasks, compared to baseline, is shown in the Grand Averages (see Fig. A.5 in Appendix B). Fifty-eight percent of all participants had a "typical pattern" of lateralization with Mental Rotation and Chimeric Faces lateralized to the right hemisphere, and Word Generation to the left hemisphere. For more information on the distribution of participants over the pattern of lateralization across all tasks, see Table A.3 in Appendix B. The average lateralization index per sex is shown in Table 1. Boys were significantly stronger lateralized to the right hemisphere for the Mental Rotation task than girls, but there is no sex difference for the other tasks.

Table 2
Results of the linear mixed model analyses for the effects of prenatal testosterone (T) and sex for all participants, for the effects of prenatal and pubertal testosterone for the girls, and for the effects of prenatal and pubertal testosterone with and without pubertal stage as a covariate for the boys.

	LI Mental Rotation					LI Chimeric Faces					LI Word Generation				
	n=57					**n=56**					**n=58**				
All participants	F	df	B	Beta	*p*	F	df	B	Beta	*p*	F	df	B	Beta	*p*
Prenatal T	0.2	56.6	-0.96	-0.59	0.281	1.3	55.9	-1.09	-0.67	0.231	1.4	55.2	-1.35	-0.82	0.131
sex	3.9	56.6	-2.36	-0.77	0.054	0.8	56.3	-1.08	-0.03	0.381	0.7	55.3	-0.96	0.65	0.423
Prenatal T * sex	1.8	56.6	1.43	0.87	0.186	0.8	55.9	0.95	0.58	0.388	1.8	55.2	1.45	0.88	0.180
Girls	**n=27**					**n=27**					**n=29**				
Prenatal T	0.0	27.1	-0.38	-0.44	0.835	0.1	27.0	-0.49	-0.43	0.798	0.0	27.5	-0.17	-0.58	0.930
Pubertal T	0.7	27.2	108.05	0.73	0.408	0.0	27.0	23.13	-0.09	0.870	0.4	27.8	85.57	0.21	0.549
Prenatal T * Pubertal T	0.2	27.2	-54.50	-0.22	0.669	0.1	27.1	-44.95	-0.18	0.745	0.5	27.6	-92.87	-0.37	0.507
Boys	**n=30**					**n=29**					**n=29**				
Prenatal T	7.1	30.5	-4.08	-0.11	**0.012**	2.8	29.1	-2.99	-0.33	0.103	0.3	28.0	-0.87	0.02	0.590
Pubertal T	8.7	30.3	-44.71	0.32	**0.006**	1.9	29.4	-24.28	0.43	0.180	1.0	27.9	-16.06	-0.33	0.317
Prenatal T * Pubertal T	9.9	30.3	32.62	1.28	**0.004**	2.7	29.2	20.08	0.79	0.109	0.5	28.0	7.65	0.30	0.487
Boys - incl. pubertal stage	**n=30**					**n=29**					**n=29**				
Prenatal T	10.9	30.2	-4.98	-0.22	**0.002**	5.5	29.1	-4.11	-0.46	**0.026**	2.4	27.8	-2.26	-0.15	0.132
Pubertal T	13.5	30.0	-57.17	0.06	**0.001**	4.8	29.4	-39.76	0.11	**0.036**	5.4	27.8	-34.99	-0.71	**0.028**
Prenatal T * Pubertal T	14.2	30.1	38.57	1.51	**0.001**	5.3	29.2	27.48	1.08	**0.028**	2.9	27.9	16.75	0.66	0.102
pubertal stage	3.9	29.2	0.38	0.60	0.058	4.2	28.7	0.47	0.73	**0.049**	9.2	28.3	0.58	0.91	**0.005**

3.3. The effect of prenatal testosterone and sex on lateralization

The model with only prenatal testosterone and sex revealed no significant effects of prenatal testosterone, sex, or the interaction, on the lateralization index of all tasks (see Table 2–All participants).

3.4. The effect of prenatal and pubertal testosterone on lateralization

The model with prenatal testosterone, pubertal testosterone, and their interaction, was analyzed for each sex separately.

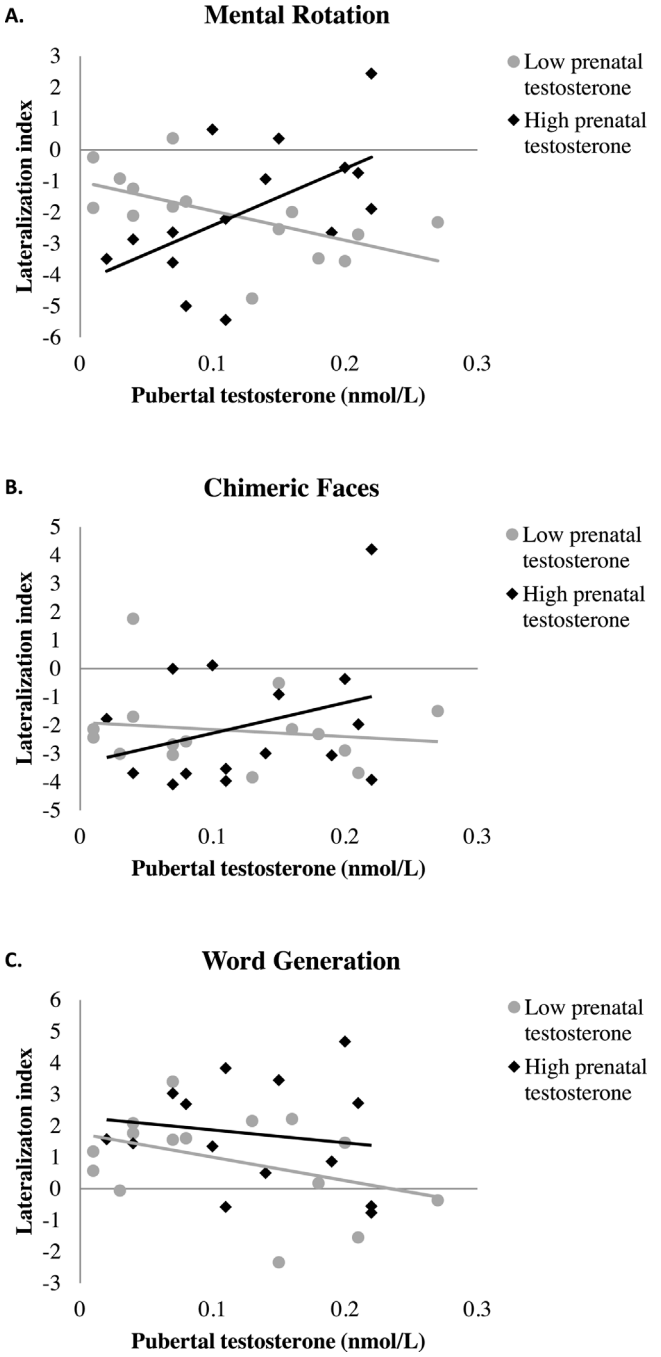

Fig. 2. The effect of pubertal testosterone on the lateralization index for the low (grey dots, grey line) and high prenatal testosterone group (black diamonds, black line). A. Mental Rotation; B. Chimeric Faces; C. Word Generation.

For girls, there were no significant effects of prenatal testosterone, pubertal testosterone, or the interaction between these two on lateralization of Mental Rotation, Chimeric Faces, or Word Generation (Table 2 – Girls). For boys, the model was first performed without pubertal stage as predictor (Table 2 – Boys), to enable comparison with girls. This revealed that in boys all predictors significantly affected lateralization of Mental Rotation. Since the model with pubertal stage as a covariate yielded a better AIC, we focus on the outcomes of this model (Table 2 – Boys incl. pubertal stage). For Mental Rotation and Chimeric Faces all effects of prenatal and pubertal testosterone are now significant, while for Word Generation only pubertal testosterone significantly affects lateralization. For all tasks, pubertal stage is positively associated with leftward asymmetry.

For Mental Rotation and Chimeric Faces, both being right-lateralized tasks, there is a significant interaction effect between prenatal and pubertal testosterone, making it difficult to interpret the main effects. We therefore performed the same analysis with standardized independent variables, and only the interaction effects remained highly significant for Mental Rotation (prenatal testosterone $p = 0.440$, pubertal testosterone $p = 0.833$, interaction $p < 0.001$, pubertal stage $p = 0.058$) and Chimeric Faces (prenatal testosterone $p = 0.168$, pubertal testosterone $p = 0.742$, interaction $p = 0.028$, pubertal stage $p = 0.049$).

To visualize the interaction effects, the boys were divided in a “low prenatal testosterone group” and a “high prenatal testosterone group”, see Fig. 2A–C. For the low prenatal testosterone group, pubertal testosterone relates to stronger lateralization towards the right hemisphere for Mental Rotation ($B = -11.69$, $SE = 4.81$, $p = 0.028$), while for the high prenatal testosterone group, pubertal testosterone relates to weaker lateralization towards the right hemisphere ($B = 14.36$, $SE = 6.68$, $p = 0.048$; Fig. 2A). Pubertal stage has no significant effect on lateralization of Mental Rotation in the low ($B = 0.19$, $SE = 0.27$, $p = 0.714$) or high prenatal testosterone group ($B = 0.33$, $SE = 0.27$, $p = 0.242$). For the Chimeric Faces task, the influence of pubertal testosterone on lateralization in both groups is similar to the Mental Rotation task, but not significant (low prenatal testosterone group: $B = -4.46$, $SE = 6.47$, $p = 0.501$; high prenatal testosterone group: $B = 9.57$, $SE = 7.58$, $p = 0.227$; Fig. 2B). Again, pubertal stage has no significant effect on brain lateralization for both groups (low prenatal testosterone: $B = 0.11$, $SE = 0.35$, $p = 0.754$; high prenatal testosterone: $B = 0.48$, $SE = 0.30$, $p = 0.133$).

For the Word Generation task, being a left lateralized function, but there is a significant negative relation between pubertal testosterone and the lateralization index: more pubertal testosterone is related to weaker lateralization index. To be able to compare the outcomes of Word Generation to Mental Rotation and Chimeric Faces, we also depicted and assessed the effects of pubertal testosterone and pubertal stage for both prenatal groups separately (Fig. 2C). The main effect of pubertal testosterone seems to be driven by the low prenatal group. For the low prenatal group, pubertal testosterone is related to weaker lateralization to the left hemisphere (or: stronger to the right hemisphere) ($B = -18.00$, $SE = 5.90$, $p = 0.009$), and pubertal stage has a reversed effect ($B = 0.81$, $SE = 0.32$, $p = 0.025$). For the high prenatal group, there is no significant effect of pubertal testosterone ($B = -5.49$, $SE = 5.95$, $p = 0.371$) or pubertal stage ($B = 0.37$, $SE = 0.24$, $p = 0.137$).

4. Discussion

This is the first study combining prenatal amniotic fluid testosterone levels with pubertal saliva testosterone levels in the same participants. An important finding of our study is that testosterone exposure is related to brain lateralization of cognitive tasks in boys at 15 years of age, but only if prenatal and pubertal levels were both taken into account. The effects of testosterone were task dependent. Because testosterone

levels increase in puberty in boys (Ober et al., 2008; this study), we added pubertal stage as a covariate to control for duration of exposure to elevated testosterone, which increased the explanatory power of the statistical model for boys. Pubertal stage increased leftward asymmetry for all tasks. The fact that in girls testosterone concentrations, especially in puberty, were very low and showing little variation may account for the non-significant effects for this sex and restricts interpretation. Nonetheless, we believe it is valuable to include the analyses for girls, as their low testosterone levels provide biologically relevant information. The results did not change when time of day of saliva collection was included, or when the analyses were restricted to right-handers.

In the analysis with only prenatal testosterone and sex as effects, we did not find any significant effects, which is in accordance with the study of Mercure et al. (2009) − the only other study that investigated the effect of prenatal testosterone in amniotic fluid on lateralization measured at the brain level. It is in contrast to the amniotic fluid studies using behavioural methods to assess lateralization (Grimshaw et al., 1995; Lust et al., 2010). These amniotic fluid studies were all performed in pre-pubertal children. There is one study investigating the effect of perinatal testosterone levels (measured in umbilical cord blood at birth) on lateralization measured with fTCD at 22 years of age (Hollier et al., 2014). In this study, no main effect of perinatal testosterone on lateralization of Word Generation or Visuospatial Memory was found, which is in line with our study when the analysis was performed without the pubertal testosterone levels in the model. In the following discussion we will focus on the outcomes of the model with the pubertal testosterone levels included. This will mainly be based on the outcomes for boys, as for girls we did not find significant effects of testosterone on lateralization.

4.1. Mental rotation and chimeric faces tasks

For the two right hemispheric tasks, the Mental Rotation and the Chimeric Faces task, we found a highly significant interaction effect between prenatal and pubertal testosterone in boys. We hypothesized that prenatal testosterone would strengthen the effect of pubertal testosterone. However, the interaction effect cannot simply be explained by prenatal testosterone upregulating the sensitivity for pubertal testosterone (Nelson, 2005). In the boys with low prenatal testosterone levels, pubertal testosterone increased the strength of lateralization for these two tasks, while in the high prenatal testosterone group, pubertal testosterone decreased the strength of lateralization. Analysis per prenatal group revealed that these effects were significant for the Mental Rotation task, but not for the Chimeric Faces task. Nevertheless, the direction and magnitude of the effect sizes is comparable between both tasks.

To understand this interaction effect, it is important to realize that an effect of hormone exposure on brain lateralization requires an asymmetrical distribution or efficiency of the relevant hormone receptors, or of subsequent downstream processes. Based on our data, we hypothesize that the distribution of androgen receptors (AR) differs between both prenatal testosterone groups, resulting in a different effect of pubertal testosterone on brain lateralization. The idea that AR distribution may be lateralized in the human brain is supported by the fact that in the brain of fetal rhesus monkeys AR distribution is lateralized (Sholl and Kim 1990). Interestingly, this is only the case for males and not for females, and the direction of lateralization depends on the brain area: more androgen receptors in the right frontal lobe, and in the left temporal lobe. Unfortunately, there is − to the best of our knowledge − no literature describing the AR distribution in the human brain. If the AR distribution is indeed lateralized in the brain areas involved in Mental Rotation and Chimeric Faces, and in what direction, remains to be investigated.

Besides our hypothesis that the AR distribution plays a role in the development of functional brain lateralization, the literature points also to a role for AR efficiency. Raznahan et al. (2010) investigated the relation between the efficiency of the androgen receptor and grey matter thickness in the brain. Remarkably they found a specific interaction effect with age in the intraparietal sulcus and the inferior parietal lobule, areas especially involved in Mental Rotation: before puberty the cortex is thinner in the high efficient androgen receptor group than in the low efficient AR group, but after puberty the difference is reversed and this effect was only found for boys, not for girls. Importantly, this interaction effect was especially pronounced in the right hemisphere. If we link this interaction effect to the interaction effect we found for Mental Rotation and Chimeric Faces in our male sample, we can hypothesize that our low prenatal testosterone group may have a more efficient androgen receptor type (explaining a weaker lateralization before and a stronger lateralization after puberty) and that our high prenatal testosterone group might have a less efficient androgen receptor type (explaining the opposite pattern). Indeed there is data that testosterone levels are lower in participants with the higher efficient androgen receptor, probably as a result of negative feedback of the androgen receptor on testosterone production, both in adults and prenatally (Crabbe et al., 2007; Krithivas et al., 1999; Manning et al., 2003; Stanworth et al., 2008; but see Eisenegger et al., 2016), supporting our explanation.

4.2. Word generation task

For the Word Generation task, pubertal testosterone decreased the strength of lateralization in boys. We did not find an effect of prenatal testosterone, or an interaction effect between prenatal and pubertal testosterone. This is in contrast to our hypothesis that testosterone would strengthen lateralization both prenatally and in puberty, for example via pruning of the corpus callosum, as postulated by Witelson and Nowakowski (1991). However, Chavarria et al. (2014) reported that pubertal testosterone increases the thickness of the corpus callosum by increasing the myelin sheet and the thickness of the axons. This would result in more capacity for communication between the hemispheres, and therefore decreased lateralization like we found for Word Generation. Thus, this may explain the main effect of pubertal testosterone in our data.

There is no literature on the effect of pubertal testosterone on lateralization of language, but there are some studies investigating the effect of salivary testosterone levels in adulthood. In contrast to our study, Papadatou-Pastou and Martin (2017) found that adult testosterone increased the strength of lateralization of Word Generation measured with fTCD. However, the majority of the participants were left-handed, and there is a relation between lateralization for handedness and language (Knecht et al., 2000), possibly confounding their results. We had too few lefthanders to properly analyse the effect of handedness in this respect. There are four other studies investigating the effect of adult salivary testosterone on language lateralization measured with dichotic listening, finding that testosterone levels differed between groups based on handedness or ear advantage (Gadea et al., 2003; Moffat and Hampson, 1996; Moffat and Hampson, 2000; Papadatou-Pastou et al., 2016). However, no direct relation between adult salivary testosterone levels and language lateralization was found in these studies, suggesting that testosterone exposure in puberty − and not in adulthood − has some organizing effects on the brain.

Based on the literature and the previous study of Lust et al. (2010), we did not only expect an effect of pubertal testosterone on language lateralization, but also an effect of prenatal testosterone. Although the effect of prenatal testosterone (or the interaction) was not significant in our study, further analysis revealed that the boys with higher prenatal

testosterone levels seem to be stronger lateralized to the left hemisphere for Word Generation. This is in accordance with the finding that prenatal testosterone was positively related to left language lateralization measured with dichotic listening in the same participants at 6 years of age (Lust et al., 2010). Also, this outcome is consistent with the study of Hollier et al. (2014), who did not find a main effect of perinatal testosterone on lateralization of Word Generation at age 22, but who did find that typical left lateralization of language is more common in the high perinatal testosterone group.

4.3. Task differences

There were clear differences in the relationships between prenatal and pubertal testosterone and lateralization of the Word Generation task versus the Mental Rotation and Chimeric Faces tasks. This could be due to the fact that Word Generation task was the only left lateralized task, and that testosterone exposure did not affect this hemisphere. It could also be the case that the effect of testosterone on specifically the language areas is different from other brain areas (e.g. Lombardo et al., 2012; Raznahan et al., 2010), or that testosterone specifically affects the neural fibres of the corpus callosum connecting language areas. Chura et al. (2010) found a positive correlation between prenatal testosterone levels in amniotic fluid and rightward asymmetry of the isthmus of the corpus callosum at age 8–11 years. The isthmus projects to brain regions involved in language, emotion recognition and visuospatial cognition (Chura et al., 2010), but how prenatal testosterone affects the neural fibres of the isthmus that connect different functional brain regions needs to be investigated. Moreover, the effect of pubertal testosterone on functional subsections of the corpus callosum warrants further study as well.

Most studies on human brain lateralization, especially functional Transcranial Doppler (fTCD) studies, focus only on language tasks. In our study we compared the outcomes on three cognitive tasks. The Chimeric Faces task has not been assessed with fTCD before, and it turned out that 87.5% of participants were right-lateralized for this task (see Table A.3 in Appendix B). Further, the strongest effects of testosterone on lateralization were found for the Mental Rotation task. For all tasks, we show that the average lateralization as assessed with fTCD is in the expected direction. However, the individual differences in lateralization were much larger than previously assumed. Namely, the typical lateralization pattern for the three tasks was found in 58% of participants. The effects of sex hormones may depend on the specific lateralization pattern (see for example the analyses by Lust et al., 2011b). In the present study the number of participants was too small, but future studies may pursue this. Our results clearly indicate that using more tasks than only language tasks is useful.

4.4. Sex effects

Finally, there is a significant sex effect for lateralization on the Mental Rotation task: boys were on average stronger lateralized to the right hemisphere than girls. This is often assumed in the literature, but to our knowledge, the present study demonstrates this for the first time with lateralization measured at the brain level (instead of measuring performance only). The sex effect we found for Mental Rotation supports the Sexual Differentiation theory (Hines and Shipley, 1984). Unfortunately, we could not distinguish the effect of pubertal testosterone from the effect of sex in our sample, as there is a strong relation between testosterone and sex, and because testosterone levels showed little variation in girls. It would be interesting to study girls with elevated testosterone levels in puberty, to see if the effects we found in boys are present in girls as well. Alternatively, the effect of testosterone might not only be task-specific, but also sex-specific: Literature suggests that pubertal testosterone has a different effect on the male and female brain (Peper et al., 2011; Peper et al., 2009; Perrin et al., 2008; Raznahan et al., 2010). Moreover, estradiol is an important metabolite from testosterone, and the estrogen receptor might play a role here, and this may depend on the task and sex. For example, pubertal testosterone is positively related to grey matter density in boys, and estradiol is negatively related to grey matter density in girls (Peper et al., 2009). Finally, the estrogen receptor distribution is also lateralized in the brain, and the direction differs between males and females (Diamond, 1991; Sandhu et al., 1986). We were not able to determine estradiol levels in saliva as no mass spectrometric methods were available that are capable of reliably measuring the low estradiol levels in saliva. Also, sex hormones fluctuate during the menstrual cycle, and effects of menstrual cycle phase on lateralization have been reported (e.g. Hausmann and Güntürkün 2000; Hjelmervik et al., 2012; Hodgetts et al., 2015). Unfortunately, menstrual cycle phase could not reliably be estimated in our study, limiting the evaluation of these short term effects of sex hormones on lateralization. We recommend studying the effect of elevated pubertal testosterone in girls, the effect of estradiol in both sexes, and the role of the androgen and estrogen receptors in the development of brain lateralization in future studies.

4.5. Conclusion

To conclude, this study shows that both prenatal and pubertal testosterone affect lateralization of several cognitive tasks. Strengths of our study are that we measured prenatal testosterone directly in the amniotic fluid and in saliva, thus combining prenatal and pubertal testosterone levels in the same participants, while measuring lateralization at the brain level. In addition, whereas the results of previous studies are difficult to generalize because different tasks and methods were used, we compared the effect of testosterone on lateralization of three tasks within the same study and the same participants. For Word Generation, pubertal testosterone decreases the strength of lateralization in the left hemisphere, while for Mental Rotation and Chimeric Faces there is an interaction between prenatal and pubertal testosterone. These results indicate that the effects of testosterone, both prenatal and pubertal, are task dependent and specific for the brain areas or networks involved. Both the influence of pubertal testosterone and task specificity could explain the mixed findings in the literature, and why the influence of prenatal testosterone on lateralization is still elusive after decades of research. Our findings cannot be explained by simple models in which the brain is affected by prenatal testosterone in

a similar way for all tasks, as suggested by the Sexual Differentiation theory (Hines and Shipley, 1984), the Geschwind and Galaburda theory (1985), and the Corpus Callosum theory (Witelson and Nowakowski, 1991). We proposed an alternative potential mechanism, based on asymmetrical distribution of androgen receptors, and their differential efficiency in relation to differences in prenatal testosterone exposure. Hopefully our findings will inspire others to pursue to examine our hypothesis and to bring the intriguing field of sex and hormone dependent lateralization forward.

Declaration of interest

The authors have no conflict of interest.

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Author roles

Beking, Geuze, Kreukels and Groothuis contributed to the successful funding application and were involved in the study design, data collection and analysis, writing and revising the manuscript. Geuze and Groothuis acted as daily supervisors. Van Faassen and Kema were involved in the testosterone measurement in saliva samples.

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Appendix A

Text A.1. : Information on the Chimeric Faces stimuli

The Chimeric Faces task is an interesting task to study lateralization. Unfortunately, most Chimeric Faces stimulus sets have a low resolution, are in black-and-white, not freely available, or have a limited amount of stimuli. There is need for a modern version of the Chimeric Faces stimuli, using the improvements of current photo editing software. We created a new Chimeric Faces stimulus set based on the open accessible KDEF-stimulus set (Lundqvist et al., 1998). Twenty pictures (10 males; 10 females) with the highest hit rate on happiness, and 20 pictures (10 males; 10 females) with the highest hit rate on angriness were selected (based on (Goeleven et al., 2008) and contact with the authors), together with the corresponding neutral pictures. The left half of the happy/angry picture was used, together with the right half of the neutral picture, to compose the new picture (dimension 562 × 762). The color and size of both halves was equalized. We made sure eyes, nose and mouth of both halves were on the same location. The transition between both halves was smoothed. Hairline and t-shirt of 1 picture were used. All stimulus design steps were executed in Photoshop. Next, the new combined picture was mirrored to make a stimulus pair in E-Studio, in which we arranged the stimulus pair vertically in the middle of the screen (see Fig. A.2 in Appendix B). We made sure participants were positioned in the middle of the screen, so the left side of the stimulus would correspond to the left visual half field, and vice versa. The stimuli we created and used are accessible via www.KDEF.se.

Appendix B

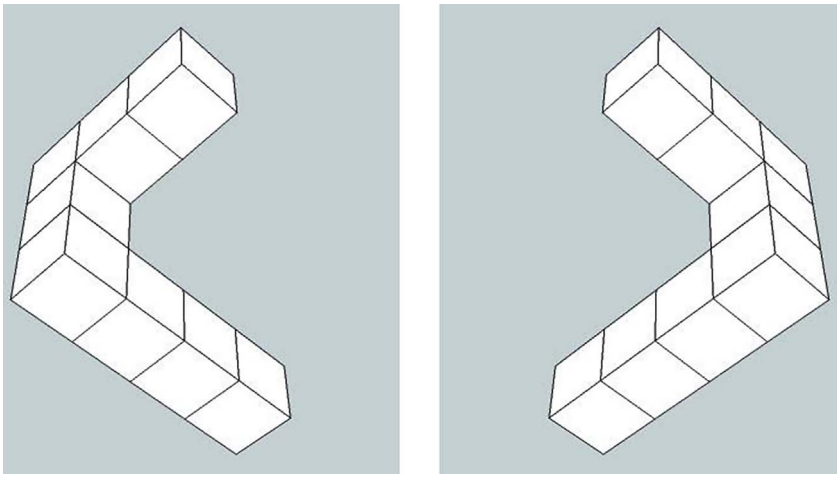

Fig. A.1. Example of a Mental Rotation stimulus.

Fig. A.2. Example of a Chimeric Faces stimulus.

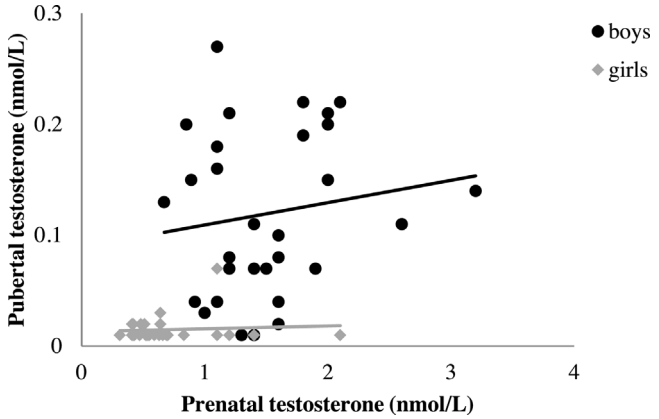

Fig. A.3. Correlation between prenatal and pubertal testosterone levels for boys (black dots, black line) and girls (grey diamonds, grey line).

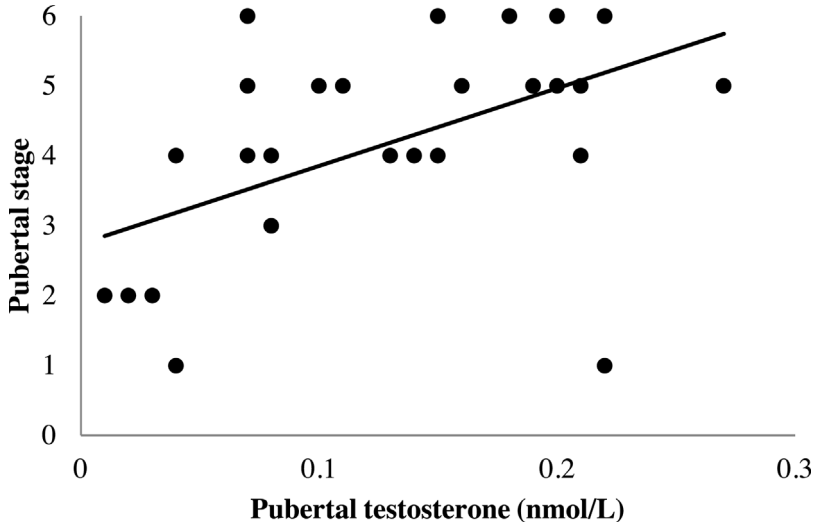

Fig. A.4. The relation between pubertal testosterone level and pubertal stage in boys.

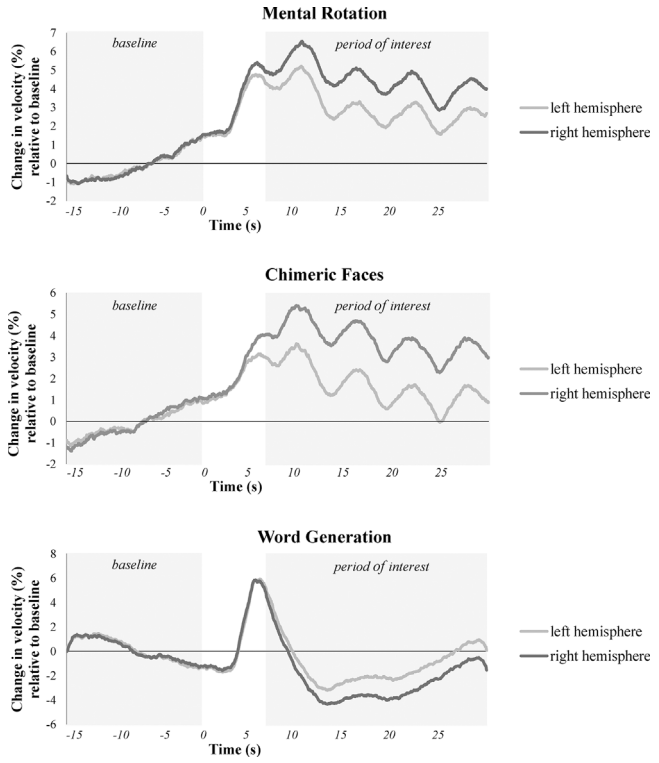

Fig. A.5. Grand averages of fTCD measure by task.

Table A.1
Results of the linear mixed model analyses for the effects of prenatal testosterone (T) and sex for all participants, for the effects of prenatal and pubertal testosterone for the girls, and for the effects of prenatal and pubertal testosterone with and without pubertal stage as a covariate for the boys. The lateralization index is based on the 2 s interval around the maximum difference between left- and right activation during task relative to baseline.

	LI Mental Rotation					LI Chimeric Faces					LI Word Generation				
	n=56					**n=56**					**n=58**				
All participants	F	df	B	SE	*p*	F	df	B	SE	*p*	F	df	B	SE	*p*
Prenatal T	3.0	55.4	4.42	2.56	0.091	1.3	55.8	-2.08	1.50	0.173	2.1	53.1	-1.97	1.29	0.132
sex	4.5	55.4	4.40	2.08	**0.039**	1.1	56.4	-2.14	2.05	0.301	0.2	53.5	-0.72	1.75	0.680
Prenatal T * sex	3.4	55.7	-3.38	1.83	0.070	1.3	55.8	2.05	1.82	0.264	1.1	53.1	1.67	1.56	0.289
Girls	**n=27**					**n=27**					**n=29**				
Prenatal T	0.3	27.4	-1.77	3.14	0.577	0.1	27.2	-1.05	3.18	0.744	0.0	25.9	0.34	2.66	0.900
Pubertal T	0.5	27.7	152.54	224.09	0.502	0.1	27.2	55.78	237.14	0.816	0.9	27.3	181.46	192.47	0.354
Prenatal T * Pubertal T	0.1	27.9	-59.92	220.17	0.788	0.1	27.3	-79.85	231.05	0.732	1.0	26.8	-185.55	188.23	0.333
Boys	**n=29**					**n=29**					**n=29**				
Prenatal T	5.6	28.4	-6.49	2.75	**0.025**	1.7	29.2	-3.91	3.01	0.204	0.3	27.5	-1.47	2.55	0.569
Pubertal T	7.1	28.2	-72.67	27.27	**0.013**	1.0	29.5	-30.44	29.88	0.317	0.7	27.4	-21.82	25.25	0.395
Prenatal T * Pubertal T	8.2	28.4	53.13	18.52	**0.008**	1.7	29.3	27.05	20.53	0.198	0.3	27.5	9.61	17.40	0.585
Boys - incl. pubertal stage	**n=29**					**n=29**					**n=29**				
Prenatal T	8.5	28.2	-7.87	2.70	**0.007**	2.7	29.2	-5.05	3.09	0.112	2.6	27.5	-3.74	2.33	0.120
Pubertal T	10.9	27.9	-91.88	27.78	**0.003**	2.1	29.5	-46.26	32.01	0.159	4.7	27.6	-52.38	24.14	**0.039**
Prenatal T * Pubertal T	11.7	28.2	62.28	18.18	**0.002**	2.7	29.3	34.62	21.00	0.110	2.4	27.6	24.40	15.87	0.135
pubertal stage	3.4	28.0	0.61	0.33	0.077	1.4	28.5	0.48	0.40	0.244	9.2	28.2	0.93	0.31	**0.005**

Table A.2
Results of the linear mixed model analyses on only the right-handed participants for the effects of prenatal testosterone (T) and sex for all participants, for the effects of prenatal and pubertal testosterone for the girls, and for the effects of prenatal and pubertal testosterone with and without pubertal stage as a covariate for the boys.

	LI Mental Rotation					LI Chimeric Faces					LI Word Generation				
	n=44					**n=42**					**n=43**				
All participants	F	df	B	SE	*p*	F	df	B	SE	*p*	F	df	B	SE	*p*
Prenatal T	0.2	44.6	-0.97	1.39	0.700	1.1	41.9	-1.60	1.39	0.305	0.7	43.0	-1.34	1.23	0.397
sex	2.4	43.9	-2.31	1.48	0.126	0.7	42.2	-1.25	1.50	0.408	1.1	42.2	-1.35	1.31	0.308
Prenatal T * sex	0.8	44.6	1.35	1.54	0.388	1.0	42.9	1.58	1.55	0.312	1.2	43.0	1.51	1.37	0.276
Girls	**n=20**					**n=19**					**n=20**				
Prenatal T	0.1	20.4	-0.70	2.28	0.763	0.3	19.0	-1.28	2.33	0.589	0.0	20.2	-0.46	2.14	0.832
Pubertal T	0.6	20.1	102.57	130.03	0.439	0.0	19.0	-8.64	140.45	0.952	0.1	20.0	36.40	121.62	0.768
Prenatal T * Pubertal T	0.1	20.2	-47.60	128.25	0.714	0.0	19.1	-10.90	137.22	0.938	0.2	19.9	-51.42	119.67	0.672
Boys	**n=24**					**n=23**					**n=23**				
Prenatal T	8.9	24.4	-5.77	1.94	**0.006**	5.3	23.2	-4.98	2.16	**0.031**	1.9	22.1	-2.67	1.93	0.180
Pubertal T	9.8	24.3	-61.56	19.66	**0.004**	4.1	23.3	-44.28	21.92	0.055	3.4	22.2	-36.08	19.54	0.078
Prenatal T * Pubertal T	10.9	24.3	46.63	14.11	**0.003**	5.5	23.3	37.10	15.76	**0.027**	2.6	22.2	22.48	14.07	0.124
Boys - incl. Pubertal stage	**n=24**					**n=23**					**n=23**				
Prenatal T	9.1	24.8	-5.76	2.72	**0.006**	3.7	23.1	-4.25	2.22	**0.068**	2.3	21.9	-2.67	1.77	0.146
Pubertal T	10.6	24.2	-63.62	1.91	**0.003**	2.8	23.3	-38.21	22.91	**0.109**	5.1	22.1	-40.93	18.11	**0.034**
Prenatal T * Pubertal T	10.8	24.3	45.71	19.50	**0.003**	3.6	23.1	30.91	16.32	**0.071**	2.5	21.9	20.57	12.95	0.127
Pubertal stage	0.7	24.6	0.23	0.27	0.399	0.4	22.8	-1.60	1.63	**0.790**	4.1	22.6	0.52	0.26	0.054

Table A.3
Lateralization patterns.

pattern	MR	CF	WG	% (n)
Expected pattern	R	R	L	58% (32)
Right hemispheric	R	R	R	18% (10)
Left hemispheric	L	L	L	7% (4)
Mirrored pattern	L	L	R	4% (2)
Other patterns	L	R	R	5% (3)
	L	R	L	5% (3)
	R	L	L	2% (1)
right lateralized	79% (45)	87.5% (49)	26% (15)	
left lateralized	21% (12)	12.5% (7)	74% (42)	

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